

CHEMICAL METHODS OF MEASURING DDT¹

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Advances in chemical methods for the control of insects have resulted from application of the techniques of chemistry and entomology to the problems of insect control. Nowhere is this better exemplified than in the development of DDT. From the entomological point of view, one of the most important contributions from chemistry is the development of quantitative methods for measuring DDT. These methods offer a valuable tool to the research entomologist and they are finding many applications in entomological research.

With the object of extending the entomologist's acquaintance with chemical approaches to his problems, this paper reviews the role of chemical methods for measuring DDT in insecticide research, and discusses the principles and salient features of some of these methods.

THE ROLE OF CHEMICAL METHODS IN DDT STUDIES

The simplest form of the common proposition in insecticide research is: How much insecticide is required to kill a certain insect? The condition "how much" requires a quantitative definition; but the result, "dead" or "alive", is a qualitative description of the gross biological response to the insecticide. Recognition of this dual relation reveals the necessary association of quantitative chemical methods with bioassay procedures in insecticide research. Chemical methods are used to define the principal condition of such investigations—that is, the quantity of insecticide to which the insects are exposed; but their response to that quantity of insecticide can be assessed only by bioassay methods.

Both methods are involved even though only one of them is specified. Thus, the entomologist studying the effect on insects of a proprietary 5 per cent DDT formulation may not analyse the insecticide, but he assumes that the manufacturer did so and found it to contain 5 per cent DDT. Conversely, the DDT residue on apples may be determined by chemical analysis and reported as more or less than the tentative tolerance of 7 p.p.m., but this result is ultimately referable to tolerances established with experimental animals.

Bioassay methods are essential to the investigation of the insecticidal effects of DDT. There is no other way to measure "insecticidal effect" than by observing the response of the specific insect concerned. This truism needs no more demonstration than the observed resistance of the

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Mexican bean beetle and the susceptibility of the housefly to DDT. The difficulty with bioassay experiments is essentially the difficulty of defining the conditions of the experiment. The principal condition is the quantity of DDT to which the test insects are exposed. But the insect response may be greatly modified by the complex factors represented by the physical state of the insecticide, the conditions of exposure, and the sex, age, previous environment and genetic constitution of the insect itself. Obviously, bioassay procedures rest on an intricate foundation. Precision depends on the extent to which the variables can be defined and controlled.

Chemical methods of measuring DDT enable accurate definition of one of these variables—the amount of insecticide to which the insects are exposed. Information on how much insecticidally active DDT is present in formulations, residues, and biological systems is basic to investigations with DDT. Whether the investigation is concerned with testing the insecticidal value of a series of DDT formulations, with seeking the most effective dosage, or with studying the effect of environmental factors on DDT residues, an essential question is how much DDT is available to kill insects. Similarly, studies of the longevity of DDT residues are concerned with how long a deposit will retain the amount of active DDT necessary to kill insects. Quantitative determination of DDT in absolute units fixes the common variable in such studies.

It must be emphasized that the amount of DDT dispensed is not necessarily equivalent to the amount deposited; indeed, the amount deposited may not be the amount available to kill insects. Thus, in both laboratory and field tests, some of the DDT dispensed may not reach the target. This fact is generally recognized and it is a common procedure to weigh the actual deposit and to calculate, from the DDT content of the formulation, the weight of DDT deposited. The assumption is made that the deposit contains the same proportion of DDT as the initial formulation, but this may not be assumed with certainty. Even when the deposit of DDT is known with certainty, absorption by the surface of the substrate may reduce the residue available to contact by insects. Chemical determination of the amount of DDT at the time and site of insect contact provides the most reliable measure of this factor.

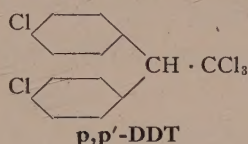
Not only the amount but also the isomeric form of DDT requires definition, and is accessible to chemical determination. The insecticidal action of DDT is mainly due to the para para isomer, and this isomer may be measured with reasonable specificity by one of the methods described in a later section. The influence of factors such as time, temperature, and chemical compounds on the insecticidal effect of DDT may be anticipated therefore by their effect on the para para isomer. Thus, thermal degradation, photodecomposition, and catalytic breakdown of DDT may be measured and ascribed as conditions modifying the insecticidal effect.

It may be objected that there is little advantage in knowing the actual amount of para para DDT to which the insect is exposed, that the essential information lies in the relation between the quantity of DDT dispensed and the observed mortality. This is true when the object of the investigation is to provide a basis for practical control measures and no further information is sought. But new approaches are opened by studies designed to evaluate the many variables in the seemingly simple relation between

observed mortality and DDT applied. Such variables, which are inevitably present in bioassay procedures, can only be evaluated when the amount and isomeric form of DDT are accurately known.

CHEMICAL METHODS OF MEASURING DDT

The term "DDT" does not refer to a specific chemical compound. There are 45 possible compounds (stereoisomeric forms not included), all of the same molecular formula, $C_{14}H_9Cl_5$ (10), and with the same generic name, dichlorodiphenyltrichloroethane. The most abundant and most toxic isomer in commercial grade DDT is the para para isomer, called p,p'-DDT for short, but which is properly named 2,2 bis (p-chlorophenyl) 1,1,1-trichloroethane. Basically, it is an ethane molecule, with its hydrogens substituted in certain places by chlorophenyl and chlorine groups. The structural formula of this para para isomer is shown below.



As commonly used, the term "DDT" refers to commercial grade DDT which contains a mixture of isomers and related compounds in which p,p'-DDT occurs in quantities from 75 per cent to 95 per cent (14), (20).

Chemical methods of measuring DDT may be placed in three groups:

Gravimetric Methods. The DDT content of the sample is measured by weighing the p,p'-DDT crystals yielded by crystallization procedure.

Volumetric Methods. The DDT undergoes molecular fission and is stoichiometrically represented by one or more of its constituent atoms. The quantity of such representatives derived from a given sample is determined by suitable titration procedures.

Colorimetric Methods. The DDT molecule is chemically altered to yield a compound with a characteristic colour, the intensity of which is proportional to the amount of DDT originally present. The colour intensity is measured photometrically at the wavelength of its maximum absorption.

The principles and salient features of methods representative of these groups follow:

Gravimetric Methods

Crystallization of p,p'-DDT

This method as described by Cristol, Hayes and Haller (9) is especially useful for assay of technical grade DDT and DDT dusts.

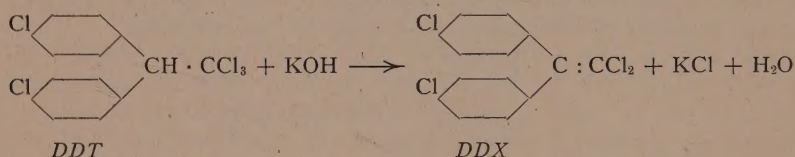
A given weight of the unknown is refluxed with a suitable amount of 75 per cent ethanol which has been saturated with p,p'-DDT at room temperature. After refluxing, the solution is cooled to room temperature whereupon the p,p'-DDT of the unknown crystallizes out quantitatively because the ethanol solution had been previously saturated with p,p'-DDT at that temperature. The crystals are suction-filtered, dried at 80°C ., weighed, and calculated as the p,p'-DDT content of the sample. A melting point determination provides a check on crystal purity (m.p. = 106°C . is satisfactory). When a small empirical correction is added, this gravimetric method is stated to be reliable to ± 1 per cent.

Labile Chlorine

Volumetric Methods

This method is based on the DDT property originally observed by Zeidler (22) in 1874. As described by Gunther (13) it is simple, rapid, and suitable for residue analysis in the range 1-15 p.p.m. (20), provided an adequate sample is available, and the nature and amount of labile chlorine-containing compounds other than DDT are known.

One of the five chlorine atoms of the DDT molecule is labile, and is detachable by hydrolysis in the presence of alkalis, involving a loss of one molecule of hydrogen chloride from the parent DDT structure. The breakdown product 2,2 bis (*p*-chlorophenyl) 1,1-dichloroethylene (DDX) is formed. Thus:



The labile chlorine atom, having become an inorganic chloride by "dehydrochlorination", is then measured by the Volhard titration method or electrometrically. Since one chlorine atom is measured, and since this represents 10 per cent of the molecular weight of DDT (354.5), results are multiplied by 10. Reproducibility is ± 0.17 mg. in the range 1.7-26 mg. (20).

Sulphur and plant materials can be important interfering factors. Baier *et al.* (1) developed a modified dehydrochlorination method for residues containing as much as 90 per cent sulphur. In a study of DDT dehydrochlorination, Wain and Martin (19) showed that reaction temperature and alkali strength lower than used by Gunther promoted analytical accuracy.

Compounds lacking labile chlorine atoms do not interfere—DDX, DDD, DDA and dichlorobenzophenone, for instance. However, other DDT isomers, hexachlorocyclohexane, chlorinated camphene, chlordane, and some other chlorinated insecticides also have labile chlorines, thus limiting the interpretation somewhat. A labile chlorine procedure has been used for total BHC assay, where only hexachlorocyclohexane was known to be present (12).

Total Chlorine

Methods based on total chlorine determination are widely used for measuring DDT residues on agricultural crops and in the determination of DDT content of sprays, dusts, and other formulations.

All five organically bound chlorine atoms are converted to inorganic chlorides by reduction with metallic sodium in benzene in the presence of isopropanol as catalyst. The resulting sodium chloride is estimated by standard titration methods, usually the Volhard method, or electrometrically. Results are multiplied by 2 since the five chlorine atoms

measured constitute 50 per cent of the molecular weight of DDT. Reproducibility of ± 0.05 mg. in the range 0.5–8.0 mg. is reported in a comparative study by Wichmann *et al.* (20).

Total chlorine methods are not specific for DDT in the presence of other chlorine-containing compounds. All DDT isomers, hexachloro-cyclohexane, 2,4-D, DDX, and similar substances will interfere. Therefore it is important to establish that the chlorine-containing material is DDT.

Carter and Hubanks (4) have shown that the rate of decomposition of DDT in residues may be measured by determining both labile and total chlorine respectively in a given sample. The ratio Labile Chlorine: Total Chlorine for undecomposed DDT is 0.20 theoretically. Values lower than this indicate DDT breakdown. This combined technique was applied in determining the effect of light (6) and temperature (11), (21) on DDT residues.

COLORIMETRIC METHODS

Xanthydrol-Pyridine-KOH Reaction

This method, described by Stiff and Castillo (17), is rapid and sensitive. In addition it is adaptable for qualitative and rough quantitative field operations (18).

DDT heated in anhydrous pyridine containing xanthydrol and solid KOH yields a red colour. The intensity of this colour at a wavelength of $520\text{ m}\mu$ is proportional to the amount of DDT present. This method is sensitive to 10 micrograms (mmg.) of DDT and is quantitative in the range 10–240 mmg. The chemistry of the test is not known.

This method does not distinguish between DDT and DDX. It is selective for DDT in the presence of DDD (7). The red colour is given by compounds having the structure $>\text{CHCX}_3$ or $>\text{C}=\text{CX}_2$ (15). The xanthydrol-pyridine-KOH reagent must be prepared daily. A number of critical features such as time, temperature, and moisture limits must be observed.

Nitration Reaction

This is usually called the Schechter-Haller method (16). The dried DDT residue is intensively nitrated, thereby yielding a tetra-nitro derivative. After isolation of the tetra-nitro DDT, involving a number of steps, it is dissolved in a known amount of benzene. Upon adding a definite volume of methanolic sodium methylate to the benzene solution a reasonably stable blue colour results, showing maximum absorption at $600\text{ m}\mu$, the intensity of which is proportional to the amount of p,p'-DDT present. This method may also be used to determine o,p'-DDT, which gives a violet-red colour with two absorption peaks at 590 and $510\text{ m}\mu$, respectively.

To date, this method is the most specific and sensitive for p,p'-DDT. While it cannot differentiate between p,p'-DDT and DDD, it can measure p,p'-DDT, o,p'-DDT, and DDA in the presence of each other. It is negative to DDX. Using this method, one of the authors (B.B.) has detected 5 mmg. in DDT residues and has found reproducibility of ± 1 mmg. in the working range 10–125 mmg. With certain modifications, Clifford (8) has adapted the method for the micro range 0–50 mmg.

A complete catalogue of colorimetric methods for DDT analysis would include a number of other tests, such as the Friedel-Crafts reaction (2); H_2SO_4 —glacial acetic reaction (5); hydroquinone— H_2SO_4 reaction (3), and the 2,4-dinitrophenylhydrazine reaction (20). They serve to illustrate the varied colour reactions to which the DDT molecule is amenable.

A word about analytical preference is in order. Each method varies with regard to specificity, sensitivity, reproducibility, and response to interfering substances (fats, waxes, plant resins, organic sulphur, chromogenic compounds, etc.) Choice of a given method must be predicated on conditions of the particular investigation, such as sample size, total residue per sample, nature and amount of substances present other than DDT, and level of reproducibility desired.

SUMMARY

Bioassay methods are indispensable to insecticidal evaluation of DDT. However, the complex of variables associated with biological assay makes accurate interpretation difficult. The principal variable is the amount of DDT present at any given time, and this is amenable to exact determination by chemical methods. Such knowledge can be useful as an absolute reference point for improving the precision of bioassay techniques.

A number of chemical methods for measuring DDT are available which provide the entomologist with procedures suited to a wide range of entomological problems. The economic and biologic importance of DDT require utilization of the most precise research methods. In this capacity chemical methods of DDT measurement are playing an increasingly important role.

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REACTION OF BARLEY VARIETIES TO SPRING FROST¹

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While spring frost cannot be listed among the more important factors limiting cereal production, it can on occasion be responsible for widespread damage. In extreme cases, losses may be so severe as to necessitate reseeding; in less severe cases the crop may be badly thinned, enabling weeds to gain the ascendancy; and in still other cases, losses may be reflected in reduced yields caused by delayed maturity forcing the crop to mature in a less favorable period of the growing season.

It is a generally accepted fact that barley is more readily damaged by spring frost than wheat. The barley breeder is cognizant of this fact and is interested in incorporating as high a degree of frost resistance as possible into his hybrid productions. He has been somewhat handicapped in this regard because of comparatively meager information available on the reaction of barley varieties to freezing temperatures, particularly in the case of those varieties developed during the past ten or twelve years. The work of Harrington (1) carried out under natural conditions, and of Platt (3) using chambers cooled by refrigeration, showed that varieties differ quite markedly in their capacity to survive freezing temperatures.

An excellent opportunity to augment the known information on frost resistance of barley varieties presented itself on the night of May 27, 1947, when, following several abnormally cool days, the temperature dropped at many points in western Canada to well below the freezing-point. Extensive damage to crops and gardens resulted. Included in the area covered by this frost were the Dominion Experimental Stations at Swift Current, Sask., and Scott, Sask.; and the Dominion Experimental Farm at Brandon, Man. At all three points, careful notes were taken on frost damage to cereal varieties. A comparison of these data revealed that a particularly good differential reaction was indicated in the case of barley and at the same time a decision was arrived at to summarize the data for publication.

METEOROLOGICAL CONSIDERATIONS

A feature of the frost in question was its long duration. At many points, freezing temperatures set in an hour or two before sunset and continued until several hours after sunrise the following morning. It is also noteworthy that this frost was immediately preceded and followed by several other frosts of lesser intensity.

Temperature Data at Scott, Sask.

The following minimum temperatures were recorded at Scott: May 25, 24.5° F.; May 26, 17.0° F.; May 27, 12° F.; and May 28, 15° F. The maximum temperature recorded on May 27 was 40° F. The temperature dropped to freezing by 5 p.m. and continued freezing until 9 a.m. of

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the morning of May 28—a duration of sixteen hours. This frost was considered to have caused the most damage but there was no doubt that four continuous nights of frost aggravated the situation.

Following the frost, comparatively warm weather with maximum temperatures ranging around 65°-70° F. occurred and the first shower (.36 inches) was recorded on June 4—one week after the frost. Recovery of all barley varieties was fairly good but the later maturing types suffered in comparison with early maturing types by being forced to head out during a dry, hot period, which prevented the heads from emerging from their sheaths in some instances.

Temperature Data at Swift Current, Sask.

Frosts were recorded on three successive nights as follows: May 26, 9° F.; May 27, 14° F.; and May 28, 5° F. The maximum temperature on May 27 was 44° F. It was estimated that the frost was of about twelve hours' duration. Conditions following the frost were favorable for rapid recovery. Temperatures remained relatively low and a shower of rain (.20 inches) was received on May 30. All varieties apparently recovered completely.

Temperature Data at Brandon, Man.

Frost was recorded on six successive nights. The temperatures were: May 24, 21.8° F.; May 25, 26.5° F.; May 26, 22.7° F.; May 27, 15.9° F.; May 28, 22.5° F.; and May 29, 30.4° F. On the night of May 27 when the bulk of the damage occurred, freezing temperatures commenced at 8 p.m. and continued throughout the night until 8 a.m. the next morning. A week of cool weather following the frost enabled the barley to make quick recovery. On June 8, approximately one inch of rain fell. The only permanent injury noted was in the case of a few varieties that had suffered over 80 per cent foliage loss. In these instances, tillering was sparse, the plants failed to regain normal vigour, and yield was noticeably reduced.

GENERAL METHODS

At all three Stations, frost susceptibility was expressed in terms of per cent foliage killed. The individual plant was used as the basis of measurement but only a single estimate was made for each plot. The readings were made within two or three days of the frost before any visible signs of recovery were evident.

While some differences existed among the several tests as to the stage of growth of the barley at the time of the frost, for the most part the plants were in what is commonly referred to as the three- to four-leaf stage.

In applying the analysis of variance method for determining significance, percentage data from the Brandon and Swift Current tests were transformed to degrees. The Scott data were not transformed since none of the percentages was particularly low or high (2).

A six replicate uniform barley test conducted at many points in Western Canada and known as the National Barley Test was caught by frost at all three Stations. Since the frost readings obtained offered an

interesting comparison of varietal reaction, these data have been grouped in a single table. In addition to the National Barley Test, notes were taken at Swift Current on the Great Plains Nursery and on eight Supplementary Barley Tests located at some distance from the Station. • At Brandon, readings were also made on the varieties in the Manitoba Co-operative Barley Test and on selections in two large hybrid tests. At Scott, frost readings were made on a group of seven varieties not represented in the National Barley Test.

National Barley Test.

EXPERIMENTAL DATA

This test is conducted under the jurisdiction of the Sub-Committee on Plant Breeding and Production of the National Barley Committee and has as its chief objective the testing of promising hybrids developed in Canada as well as new varieties that have gained prominence in the United States. Several recommended varieties are included for purposes of comparison. In 1947, the test consisted normally of twenty-five varieties. However, at some stations, extra varieties were added to meet local needs. This will explain the presence of thirty-three varieties in the Swift Current test compared with only twenty-five in the tests at Brandon and Scott.

The frost damage data compiled at the three Stations have been summarized in Table 1. It will be noted that highly significant varietal differences were established at Brandon and Swift Current, whereas at Scott these differences only exceeded the level of significance by a relatively small margin. The greatest range of varietal reaction was obtained at Brandon where actual per cent foliage damage varied from 8 per cent and 9 per cent in the case of the varieties Compana and Bay to 89 per cent in the case of the University of Alberta hybrid 43-46. A good agreement exists between the Brandon and Swift Current data. The varieties Compana, Bay, Tregal, Titan, OAC. 21, UM. 1522 and Ottawa 2526A are among the highest ranking for frost resistance at both Stations. Similarly, the three University of Alberta selections—as well as Frontier, Montcalm and Velvon 11—are among the most susceptible. The varieties Feebar and Br. 3950-1136 were rated relatively more resistant at Swift Current than they were at Brandon.

While the results obtained at Scott are in general agreement with those obtained at the other two points, there are a few exceptions. The variety Bay, which showed good resistance at Brandon and Swift Current, was one of the more susceptible at Scott. Titan was also rated as being relatively more susceptible. On the other hand, the three Brandon selections (notably Br. 3950-1239) were more resistant at Scott than at the other Stations. However, the analysis of the data shows that the frost damage at Scott was not nearly so uniform as that at the other two stations. The standard error, as well as the minimum significant difference, was considerably higher at Scott. Thus although discrepancies do occur between the Scott data and the rest, the rank of the varieties based on the minimum significant difference is not changed as much as the actual damage ratings would indicate.

With reference to the eight additional varieties grown in the Swift Current test, the high resistance shown by Vance Smyrna is noteworthy.

In fact, this variety showed the least foliage loss of the thirty-three tested at that Station. Trebi and Rex rank in resistance about equal to Titan or Tregal. The remaining varieties would be classified as "susceptible" or "moderately susceptible".

TABLE 1.—SPRING FROST DAMAGE TO BARLEY VARIETIES GROWN IN THE NATIONAL BARLEY TEST AT BRANDON, MAN., SCOTT, SASK., AND SWIFT CURRENT, SASK., IN 1947

Variety	C.A. number	Per cent foliage loss					Average
		Brandon		Scott	Swift Current		Actual
		Actual	Trans- formed	Actual	Actual	Trans- formed	
Compana	1154	8	15.7	30	20	26.6	19.3
Tregal	1150	13	20.6	22	30	33.1	21.7
Ott. 2526A	94	20	26.5	20	28	32.0	22.7
OAC. 21	1086	15	22.8	20	38	38.2	24.3
UM. 1522	41	16	23.3	24	37	37.2	25.7
Titan	1164	15	22.7	40	27	31.0	27.3
Ott. 2206B	93	21	26.9	22	45	42.1	29.3
Bay	112	9	17.3	58	22	27.7	29.7
Plush	1117	25	29.8	30	47	43.1	34.0
Br. 3957-154	101	26	30.5	25	53	46.9	34.7
Vantage	1162	21	27.1	40	48	44.0	36.3
Br. 3950-1239	102	44	41.6	18	60	50.8	40.7
Br. 3950-1136	1167	46	42.6	30	48	44.0	41.3
Glacier	1149	36	36.7	31	57	48.9	41.3
Feebar	113	32	34.2	58	38	38.2	42.7
Br. 3951-1360	1163	46	42.6	30	72	57.9	49.3
Gem	111	45	42.1	59	50	45.0	51.3
Velvon 11	1151	49	44.5	45	67	56.9	53.7
Newal	1089	55	47.9	55	62	51.8	57.3
UM. 856	114	40	39.2	68	65	53.8	57.7
Montcalm	1135	51	45.5	56	70	57.0	59.0
Frontier	110	57	48.9	57	70	57.0	61.3
U of A 42-24	96	83	65.9	60	78	62.6	73.7
U of A 43-10	95	83	65.9	59	82	65.0	74.7
U of A 43-46	97	89	71.2	62	90	73.3	80.3
Vance Smyrna	134	—	—	—	10	18.4	—
Trebi	753	—	—	—	27	30.9	—
Rex	1113	—	—	—	30	33.2	—
Warrior	1144	—	—	—	48	44.1	—
Br. 3950-1986	135	—	—	—	52	46.0	—
Prospect	1140	—	—	—	57	48.9	—
Ab. 36-1991	136	—	—	—	65	53.8	—
Atlas	702	—	—	—	65	53.8	—
<i>Statistics:</i>							
Mean	—	—	37.3	34.7	—	45.2	—
SE in per cent	—	—	4.50	17.3	—	3.65	—
Min. Sign. Diff.	—	—	4.75	19.0	—	4.66	—
F value	—	—	74.80	3.41	—	54.32	—
Five per cent pt.	—	—	1.60	1.60	—	1.14	—

NOTE: UM—University of Manitoba.

Ott—Cereal Division, Central Experimental Farm, Ottawa.

U of A—University of Alberta.

Br—Dominion Experimental Farm, Brandon, Man.

Great Plains Barley Nursery, Swift Current, Sask.

The spring frost ratings on the varieties comprising this test have been summarized in Table 2. Varieties have been arranged in ascending order of susceptibility. Each of the values given is the mean frost reading from three replicates. The statistical analysis reveals that the varieties Munsing and Titan, while not differing significantly among themselves, suffered significantly less foliage damage than did any of the other varieties in the test with the exception of Tregal. On the other hand, the Velvon selections, as a group, showed high susceptibility. The uniform results obtained in the case of these Velvon strains reflect favorably on the reliability of the data presented.

TABLE 2.—SPRING FROST DAMAGE TO BARLEY VARIETIES COMPRISING THE GREAT PLAINS NURSERY, GROWN AT SWIFT CURRENT, SASK., 1947

Variety	C.I. number	Per cent of foliage loss	
		Actual	Transformed
Munsing	6009	23	28.8
Titan	7055	23	28.8
Tregal	6359	30	33.0
Nebr. 381162	7114	40	39.1
Nebr. 383999	7261	43	41.1
Nebr. 383576	7263	43	41.1
Nebr. 383962	7262	47	43.1
36Ab. 2031	7152	47	43.1
Atlas 46	7323	47	43.1
SD. 385	7260	47	43.1
Spartan	5027	50	41.7
SD. 252	7250	53	46.9
Beecher	6566	60	50.8
Gem	7243	60	50.8
Velvon BC ₄ -68	—	63	52.8
36Ab. 6127	7008	63	52.8
Flynn 37	5918	67	54.8
Velvon BC ₄ -15	—	67	55.1
Club Mariout	261	70	56.8
H.C. 41-94	7258	70	56.8
Flynn 1	5911	70	57.0
Velvon BC ₄ -12	—	73	59.0
Velvon BC ₄ -51	—	73	59.0
Velvon 313	—	73	59.0
Velvon 11	7088	73	59.0
Velvon BC ₄ -6	—	77	61.2

Mean—48.9 SE in %—5.75 Min. Sig. Diff.—7.95 F value—11.63 5 per cent point—1.74

Supplementary Barley Test, Swift Current, Sask.

Notes on frost reaction were taken on a group of eight tests conducted by the Dominion Experimental Station, Swift Current, in the region served by that institution. Six of these tests comprised a uniform set of six varieties. It will be noted from the summarized data presented in Table 3 that considerable variation existed from point to point in the degree of damage that resulted. Highly significant varietal responses were demonstrated for each test and these are consistent with those already noted. In order to check statistically on the possibility of differential varietal response,

TABLE 3.—SPRING FROST DAMAGE TO BARLEY VARIETIES GROWN AT EIGHT LOCATIONS IN 1947 BY DOMINION EXPERIMENTAL STATION, SWIFT CURRENT, SASK.

Location		Per cent foliage loss resulting to						Statistics			
		Titan	Velvon 11	Tregal	Vantage	36Ab-1991	Compana	Mean	SE in %	Nec. Diff.	F value 5% point
Shaunavon	Actual	42	80	22	45	80	35	45.8	3.91	5.06	67.81
	Transformed	40.7	63.8	28.3	42.1	63.8	36.2				2.90
Valjean	Actual	68	82	50	48	88	35	52.6	2.07	3.10	145.86
	Transformed	55.3	65.9	45.0	43.6	69.6	36.2				2.90
Riverhurst	Actual	30	40	25	22	38	20	32.5	4.69	4.31	11.34
	Transformed	33.2	39.2	29.9	28.3	37.7	26.6				2.90
Vidra	Actual	66	79	44	65	78	53	53.5	1.77	2.69	77.97
	Transformed	54.2	62.9	41.6	53.8	61.8	46.7				2.90
Fox Valley	Actual	50	78	30	55	72	38	47.3	3.06	4.10	59.97
	Transformed	45.0	61.8	33.2	47.9	58.5	37.7				2.90
Kyle	Actual	42	70	30	45	65	35	43.8	3.34	4.14	42.85
	Transformed	40.7	57.0	33.2	42.1	53.8	36.2				2.90
Carmichael	Actual	25	52	22	45	50	—	38.3	4.25	4.61	28.02
	Transformed	29.9	46.5	28.3	42.1	45.0	—				3.26
Tugaske	Actual	40	42	25	22	45	—	36.0	4.52	4.61	15.71
	Transformed	39.2	40.7	29.9	28.3	42.1	—				3.26
Average	Actual	43.4	65.4	31.0	43.4	64.5	36.0				
	Transformed	42.5	54.7	34.9	41.0	52.8	36.6				

the data for the six stations having a uniform set of varieties were combined and analysed by the variance method. The Chi-square test as proposed by Bartlett (2) was applied to the six estimates of variance involved and they were found to be homogeneous (P lying between .50 and .20).

The results revealed a significant interaction between variety and Station ($F=5.27$; 5 per cent point—1.64) which would indicate that all varieties had not responded similarly at the different Stations. It was also found, however, that the variance of variety means significantly exceeded that of variety and Station interaction ($F=29.52$; 5 per cent point—2.60). It would appear, therefore, that, despite some differential response, varietal reaction generally was consistent enough to enable the establishment of definite variety differences. It may be assumed that the resistance shown by Compana and Tregal, and the susceptibility by Velvon 11 and 36Ab-1991, are definite variety characteristics.

Manitoba Co-operative Barley Test, Brandon, Man.

This test originated at the Plant Science Department, University of Manitoba, and comprised, in addition to the seven recommended varieties for Manitoba, a group of hybrids that have shown promise from a malting standpoint. Since this paper is concerned primarily with the frost reaction of named varieties, the hybrid types have not been included in the summary of results given in Table 4.

TABLE 4.—SPRING FROST INJURY TO SEVEN BARLEY VARIETIES GROWN IN THE MANITOBA CO-OPERATIVE BARLEY TEST, BRANDON, 1947

Variety	C.A. number	Per cent of foliage loss*	
		Actual	Transformed
OAC. 21	1086	13	21.0
Titan	1164	14	21.9
Vantage	1162	17	24.3
Plush	1117	17	24.3
Sanalta	1088	18	26.4
Wisconsin 38	758	24	29.3
Montcalm	1135	34	35.6

Gen. Mean—26.1 SE in %—3.80 Nec. Diff.—2.80
F value—25.39 5 per cent point—2.51

* Values given represent mean from five replicates.

While the general level of damage is lower than in the case of the other tests discussed, some highly significant differences exist. The superior frost resistance of OAC. 21 compared with that of Montcalm is strikingly brought out. The reaction of the varieties Sanalta and Wisconsin 38 may be of interest since these varieties have not appeared in previous tables.

Observations on Frost Damage to Barley Hybrids at Brandon, Man.

Frost readings were made in the case of two hybrid barley tests, totalling 130 selections. The barley in these tests had not progressed far beyond the two-leaf stage and damage appeared to be more severe than it

was in the case of the National Barley and Manitoba Co-operative Barley Tests which had been sown considerably earlier. Many of the hybrids were selections from Newal \times Peatland derivatives crossed on to such varieties as OAC. 21, Mensury Ott. 60, Plush and Trebi. As might be expected, a relatively wide range of reaction was found. On the whole, however, the hybrid selections exhibited considerably more susceptibility than did the standard varieties OAC. 21, Titan and Plush. A group of Velvon selections, similar to that grown at Swift Current (*see Table 2*) proved highly susceptible. From a casual examination of the data, there appeared to be little relationship between the amount of frost damage suffered by the different hybrids and their parental make-up.

One observation is presented as a matter of interest. One group of 45 hybrids possessed a rather complicated parentage involving both OAC. 21 and Montcalm. Without exception these hybrids proved as susceptible as the Montcalm parent. On the other hand, the OAC.21 check plots could be picked out readily at some distance because of considerably less damaged foliage.

Additional Data from the Scott Station

At Scott, frost damage rates were recorded on a hybrid test in which had been included seven standard varieties. The amount of leaf loss resulting to these seven varieties was as follows: Warrior—10 per cent; Regal—22 per cent; Prospect—35 per cent; Law—38 per cent; Velvon—40 per cent; Hannchen—43 per cent; and Rex—50 per cent. The resistance shown by Warrior is noteworthy.

Consistency of Results

DISCUSSION

It is evident from an examination of the data presented that many barley varieties differed significantly in their capacity to endure freezing temperatures without injury. A feature of the results obtained was the relatively consistent reaction shown by varieties in the different tests. For example, in no instance were the varieties Montcalm, Velvon, Newal, and 36Ab. 1991 classified as anything but susceptible. On the other hand such varieties as OAC. 21, Compana and Tregal consistently fell into the resistant group. Several varieties, including Plush, Vantage and Titan, varied in their reaction from moderately resistant to resistant but in no case could they be termed susceptible.

The failure of a few varieties to react the same at Scott as they had at Brandon and Swift Current may perhaps be explained by the fact that exceptionally severe frost conditions occurred at that point. Platt (3) noted that the reactions of certain barley varieties were dependent upon the freezing temperatures to which they were exposed.

Frost Resistance in the Barley Improvement Programme

As pointed out in the introduction of this paper, spring frost resistance is not a sufficiently important character to warrant top ranking priority in any barley breeding programme. The inherent tenderness of the barley crop to low spring temperatures, however, is a challenge to barley breeders to eliminate in so far as possible yet another of the natural hazards of

barley production. There is no doubt that this character has more significance now than it did some years ago owing to a trend toward earlier seeding. In some sections of the Open Plains Area, where barley has not been a profitable crop, the barley is the first crop sown in order to benefit more fully from spring moisture. Obviously where such practices are followed, the use of varieties possessing a relatively high degree of cold resistance would be a safeguard against losses from freezing temperatures in the spring of the year.

SUMMARY

1. A report is given on the reaction of barley varieties in tests at the Dominion Experimental Stations, Swift Current, Sask., and Scott, Sask.; and at the Dominion Experimental Farm, Brandon, Man., to a severe and widespread frost occurring on the night of May 27, 1947.
2. Significant variety differences were obtained in the case of all tests.
3. The behaviour of the varieties in the different tests was generally consistent.
4. Among the more resistant named varieties were: Compana, Bay, Vance Smyrna, Munsing, Tregal, OAC. 21 and Titan. Relatively high susceptibility was shown by: Velvon, Montcalm, Flynn, Frontier, Newal and Gem.
5. The hybrid selections represented in the tests showed a wide range of reaction—from extreme susceptibility to a resistance about equal to that of OAC. 21.

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A PRELIMINARY EVALUATION OF SOME INSECTICIDES AGAINST IMMATURE STAGES OF BLACK- FLIES (DIPTERA: SIMULIIDAE)^{1, 5}

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INTRODUCTION

Exploratory studies of the comparative effectiveness of various insecticides against the immature stages of blackflies were carried out at Churchill, Manitoba, in June and July, 1947, as part of a wider program of studies on the biology and control of biting flies prevalent in this region. The results of other phases of the study are to be reported elsewhere.

Apart from work with miscible oils which gave consistently uneconomic control, very little previous work has been done with insecticides against immature blackflies. In 1945, Fairchild and Barreda (3) demonstrated that a number of species of *Simulium* occurring in Guatemala could be controlled by the application of a DDT emulsion to give a concentration of 0.1 parts of DDT per million parts of water for a period of one hour, and that a solution of DDT in a turpentine-kerosene mixture and a suspension of DDT in water with a wetting agent were equally effective. Garnham and McMahon (4) have reported the local eradication of *S. neavei* Roubaud in Kenya Colony in 1946, using the much higher concentrations of 2 to more than 5 parts of DDT per million parts of water for exposure periods of 30 minutes. At these latter dosages mortality among fish and other aquatic animals was considerable. Steward (6) found that, in the laboratory, 0.25 p.p.m. DDT for one hour caused almost complete mortality of *Simulium* larvae, while Gammexane gave similar results at 0.125 p.p.m.

For purposes of comparison of these various exposure times and concentrations, it may be assumed that the time-concentration curve for any given mortality approximates to an hyperbola over the small range considered (2), and hence that:

$$\text{concentration} \times \text{time} = \text{a constant.}$$

Dosages in these experiments may then be expressed as products of concentrations in parts per million and exposure times in minutes as follows:

Fairchild and Barreda (DDT).....	6	p.p.m./minutes
Garnham and McMahon (DDT).....	60 to more than 150	p.p.m./minutes
Steward (DDT).....	15	p.p.m./minutes
Steward (Gamma-BHC).....	7.5	p.p.m./minutes

It was decided to attempt to confirm these results under the conditions at Churchill, to compare the effect obtainable with some other insecticides, and finally to explore the possibility of reducing the time of exposure required. In view of the great number of streams which would have to

¹ The results herein reported were obtained through the joint efforts of the Canadian Division of Entomology on behalf of the Defence Research Board, and the U.S. Bureau of Entomology and Plant Quarantine on behalf of the Surgeon General, Department of the Army.

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be treated for effective control in an area such as that around Churchill, the ability to do this would be of great advantage. In addition, it was hoped to obtain further data on possible toxic effects on eggs and pupae.

The work may conveniently be reported in the form of sixteen experiments, two involving aerial application of insecticides, and fourteen involving direct application of insecticides to streams. The results of these experiments are reported in the following pages. In the first two direct application experiments (Experiments 3 and 4) an attempt was made to secure quantitative estimates of populations and mortalities; this was found to be impracticable, and qualitative estimates only were attempted thereafter. The predominating species of blackfly in all experiments was *Simulium venustum* Say; species of *Eusimulium* were also involved in several of the earlier experiments. Meteorological data were recorded throughout the period of these experiments, but these data and other relevant biological information are reproduced elsewhere (8).

The following insecticidal preparations were employed in this work:

DDT (Technical Grade):

Five and 10 per cent W/V solutions in fuel oil, plus Velsicol A.R. 50 as auxiliary solvent, and 0.5 per cent Williams' red dye.

Twenty-five per cent emulsion concentrate with 65 per cent xylene and 10 per cent Triton X-100.

Fifty per cent wettable powder.

Gamma-Benzene Hexachloride:

An emulsion concentrate supplied by Canadian Industries Limited containing 5 per cent gamma isomer.

Chlorinated Camphene:

Twenty-five per cent emulsion concentrate with 65 per cent xylene and 10 per cent Triton X-100.

Chlordane:

Twenty-five per cent emulsion concentrate with 65 per cent xylene and 10 per cent Triton X-100.

Ten per cent solution in Velsicol A.R. 50 and fuel oil.

Pyrethrum—piperonyl butoxide (PPB):

Dodge and Olcott's Pyrenone emulsion concentrate (T. 143) containing 10 mgm. pyrethrins and 100 mgm. piperonyl butoxide per cc.

Experiment 1

AERIAL SPRAYING EXPERIMENTS

Two streams infested with blackfly larvae flowed in part through three plots marked out for aerial spraying trials against mosquito larvae and adults. A survey of these streams was made before the spraying to ascertain its effect on the larvae. The first of the streams flowed through the first aerial spray plot only, for a distance of about 900 yards.

The first half-mile of the second stream was in the second aerial spray plot, described under Experiment 2, the second half-mile in the third aerial spray plot, and only the last half-mile in the first aerial spray plot. Eight observation stations were marked out in the first stream, and five in the last half mile of the second; at each of these stations there was a moderate to heavy larval population on the afternoon of June 27. No pupae were present at that time. The mean depth of each stream and the rate of



FIGURE 1. Aircraft (C-47) applying DDT-fuel oil spray at Churchill.



FIGURE 2. Oil slick on stream from aerial spraying.



FIGURE 3. The treatment of Eastern Creek (Experiment 3) using a gravity dispenser. Top of reservoir can be seen on the left bank.



FIGURE 4. Part of the stream treated in Experiment 4 some days before treatment.

flow were estimated at the same time, the latter by timing a surface float over a measured distance with a stop-watch to get the maximum speed, and calculating the total flow from the formula:

$$V_a = 2/3 \cdot V_m \text{ (approx.)}$$

and

$$\text{Flow} = V_a \cdot A$$

where V_m is the measured maximum speed, V_a the average speed and A the cross sectional area of the water channel. Suitably uniform sections of each stream were selected for measurement, and a number of timings taken.

Spraying (Figure 1) was carried out on the evening of June 27 from a C-47 aircraft (R.C.A.F.) equipped with cargo tanks and a vertical gravity flow discharge pipe extending below the fuselage. The temperature of the water at this time was 58° F. The material used was a 5 per cent solution of DDT in fuel oil and this was put down at a mean concentration of 0.26 lb. DDT per acre. The population of blackfly larvae was re-checked on June 28, July 21 and August 7. No living eggs, larvae, or pupae of any species of blackfly were found in the treated length of the first stream at any of these examinations. Untreated portions of the stream, and similar untreated streams in the same vicinity continued to harbour immature stages until at least August 7. In the treated length of the second stream, the only larvae found at the examination on June 28 were at station 5, where there were two or three half-grown larvae per plant, on grasses rooted in the centre of the stream. This station was very near to the upstream limit of the treated length, so that the exposure time was very short indeed, and the insecticide probably not well distributed. At the later examinations, which were made after the upper reaches of the stream had been treated as well, no living eggs, larvae, or pupae were found.

The results of these observations are summarized in Table 1. DDT concentration was estimated on the assumption that all the material falling on the surface of the stream was carried along in it, as follows:

Concentration in parts per million:

$$\frac{\text{Deposit lb. per acre} \times 10^6}{4840 \times 9 \times \text{mean depth in feet} \times 62.5}$$

In the range of exposure time given, the minimum time represents the theoretical time for the water which received the spray to flow past the highest upstream observation station; the maximum time relates likewise to the furthest downstream station. In stream 1 the highest station was about half way between the limits of the sprayed length, so that here the minimum exposure time was quite high.

Experiment 2

The second aerial spray plot covered half a mile of stream No. 2 from the source downwards. This was examined for blackfly larvae on the morning of June 30 immediately before the spraying, when 4 stations, each with a heavy to very heavy larval population were marked out. The

TABLE 1.—SUMMARY OF DATA FROM INSECTICIDAL TREATMENT OF STREAMS

Exp. No.	Date of treatment	Observed treated length of stream (yards)	Average flow in Cusecs	Insecticide formulation	Method of application	Concentration p.p.m.	Application time (minutes)	Dosage* (p.p.m./min.)	Larval population	
									Before treatment	Twenty-four hours after treatment
1	June 27	900	12.20	5% DDT in fuel oil	Aerial spray	0.095	15 to 30	1.425 to 2.85	Heavy	Nil
2	June 30	2500	10.00	5% DDT in fuel oil	Aerial spray	0.150	0 to 60	0 to 9.00	Heavy	Nil for at least 41 days
3	July 11	1400	88.00	10% DDT in fuel oil	Gravity dispenser	0.100	30	3.00	Heavy all stages	Reinfested with young larvae in 14 days
4	July 11	850	6.70	10% DDT in fuel oil	Hand sprayer	0.100	30	3.00	Moderate	Reinfested with all stages in 14 days
5	July 14	250	0.57	10% DDT in fuel oil	Medicine dropper	0.590	15	8.85	Heavy and mature	Nil after first 100 yd.
6	July 14	85	1.98	Fuel oil	Medicine dropper	1.680	15	25.20	Heavy all stages	Almost unchanged
7	July 14	300	6.30	10% chlordane in fuel oil	Medicine dropper	0.079	15	1.185	Heavy all stages	Some control for 100 yd.
8	July 14	600	120.00	50% DDT wettable powder	Portable press. sprayer	0.075	20	1.50	Very heavy	Few first 350 yd., nil beyond
9	July 14	400	1.97	10% DDT in fuel oil	Medicine dropper	0.126	5	0.63	Heavy all stages	Slight reduction
10	July 15	4400	270.00	10% DDT in fuel oil	Two pressure sprayers	0.176	15	2.64	Very heavy	Nil after first 100 yd.
11	July 18	250	0.57	25% DDT emulsion	Medicine dropper	0.490	15	7.35	Heavy	One living larva found
12	July 20	80	0.87	5% gamma-BHC emulsion	Graduate and paddle	0.100	15	1.50	Very heavy and mature	About 50% reduction
13	July 21	650	0.54	P.P.B. (Pyrenone) emulsion T.143	Graduate and paddle	0.500	15	7.50	Moderate and mature	Reduced for 150 yd. only
14	July 21	1500	1.62	25% DDT emulsion	Graduate and paddle	0.100	15	1.50	Moderate all stages	Nil
15	July 24	20 120	8.20 17.50	5% gamma-BHC emulsion	Graduate and paddle	0.190 0.089	15	2.85 and 1.335	Heavy Heavy	Few living About half
16	July 24	100	13.60	25% chlorinated camphene emulsion	Graduate	0.164	15	2.46	Very heavy	No change

* See explanation in Introduction.

temperature of the stream water was 55° F. The same insecticidal material was used in the aerial spraying of this plot, but the deposit was 0.48 lb. DDT per acre. The stream was re-examined on the same afternoon about three hours after the end of the spraying. Only a few sick-looking larvae were found, less than 1 per cent of the previous population, all of them near the origin of the streams in a poorly defined area largely under water. Many of these were hanging on silk. A further examination on the morning of July 1, water temperature 54° F., disclosed no living larvae, either in the treated stretch or below this. Subsequent examinations were made on the same dates as for stream No. 1 and with the same results.

The third aerial spraying operation, on the morning of July 3, put down a deposit of 0.26 lb. DDT per acre on the half mile stretch of stream No. 2 in between the portions treated in the first and second operations. As this stretch had been found to be without larvae two days previously, no further observations were made on it at that time. In later surveys of the stream, this stretch was found to be equally free of infestation.

DIRECT APPLICATION EXPERIMENTS

Experiments 3-16

It had been intended to conduct these experiments primarily with emulsion formulations, but in view of the results obtained in the aerial spraying experiments with cheaper and more readily obtained oil solutions, it was decided to use these and to employ emulsions only for comparison.

The first step was the selection of suitable streams. The requirements were a high population of blackfly larvae, a well defined channel presenting a reasonable length with a minimum of tributaries or distributaries, and suitable localities for flow measurement (as described in the section on the aerial spraying experiments) and insecticide application. The streams were then surveyed, the amount of material needed to give the required concentration and time of exposure calculated approximately, and the application made. Usually an observer was present in the stream during the treatment, to watch the progress of the insecticide down the stream and observe any immediate reactions of the blackfly larvae. In general, the insecticide was applied a short distance below the upstream end of the surveyed length, in order to leave a portion of the stream undisturbed for check observations. The stream was then resurveyed as nearly as possible 24 hours after the treatment and, in most experiments, again when opportunity offered after another interval of about a week or more.

Fourteen streams were treated in all, using various concentrations and exposure periods, and including at least one test of each of the five insecticides listed in the introduction. The data and results are summarized in Table 1.

The data presented in Table 1 show that DDT in fuel oil, as a wettable powder suspension, and in emulsion form gave good control of blackfly larvae at a minimum dosage of 1.5 p.p.m./min., or 1 : 10,000,000 applied for a period of 15 minutes. Fuel oil alone at 25.2 p.p.m./min., or 1 : 600,000 for 15 minutes was ineffective. Gamma-BHC, as an emulsion, at 1.5 p.p.m./min., or 1 : 10,000,000 for 15 minutes, gave about 50 per cent

control; at approximately double this dosage a high percentage reduction of infestation was obtained. Chlordane, in fuel oil, at 1.185 p.p.m./min., or 1 : 12,700,000 for 15 minutes, gave partial control for a short distance from the point of application. Chlorinated camphene, as an emulsion, was ineffective at 2.46 p.p.m./min., or 1 : 6,000,000 for 15 minutes.

Eggs of *S. venustum* appeared to be unaffected by DDT in fuel oil at a dosage of 3 p.p.m./min., or 1 : 10,000,000 for 30 minutes. This was indicated in Experiment 4, when eggs hatched uniformly from treated and untreated parts of the stream.

Pupae in treated streams also showed no obvious ill effects from the insecticide applications, except in the case of gamma-BHC. This was ascertained by the percentage emergence of adults from samples of pupae taken from treated and untreated portions of the streams. In Experiment 12 where a dosage of gamma-BHC of 1.5 p.p.m./min., or 1 : 10,000,000 for 15 minutes, was used, emergence from treated pupae of *S. venustum* was only 12 per cent as compared with 82 per cent from untreated ones.

ADDITIONAL NOTES ON EXPERIMENTS

Description of Treated Streams

Experiments 3, 15, and 16 were carried out in different parts of Eastern Creek and its tributaries. This is a substantial stream about five miles east of Churchill camp, flowing northward into Hudson Bay through tundra meadow and patches of dwarf birch and willow, from its source in an extensive area of lakes about three miles inland. Heavy infestations of larvae were present on stones and submerged vegetation when the treatments were made. A water sample taken on July 10 had a pH of 8.46; the salinity was 56 p.p.m. chlorides measured as sodium chloride. In addition to the predominant *Simulium venustum*, two species of *Eusimulium* were in the pupal form and emerging when Experiment 3 was carried out (July 11). On the occasion of Experiments 15 and 16 (July 24) the infestation was entirely of larvae.

Experiments 1, 2, 4, 7, 12, 13, and 14 were made in small streams all, except 4 and 7, flowing in a general westerly direction to the Churchill River. Number 4 (Figure 4) originates in a small lake on the tundra and flows into Hudson Bay east of Churchill, and Number 7 flows from Warkworth Creek into the Goose River. The bottoms of the streams generally contained sand, gravel, stones and boulders. Infestations ranged from moderate to very heavy. A water sample from stream 4 had a pH of 8.35 and a salinity of 143 p.p.m.

Experiments 5, 6, 9, and 11 were in drainage ditches, similar to the type shown in Figures 6 and 10. The turbulence in these channels was less than in the streams, being almost nil at the time of treatment. However, in spite of the slow rate of flow, parts of them were heavily infested with larvae and pupae of *S. venustum*.

Experiment 8 was in the Goose River, a short distance downstream from the railway bridge which crosses it. This is a large stream (Figure 7) with a bottom of large stones and boulders. Experiment 10 was carried



FIGURE 5. Stream treatment with a hand spray pump in Experiment 4.



FIGURE 6. Drainage channel, typical of those treated in Experiments 5, 6, 9, 11, and 14.



FIGURE 7. General view of Goose River, treated in Experiment 8.



FIGURE 8. General view of Warkworth Creek, treated in Experiment 10.

out in Warkworth Creek, the largest river treated (Figure 8). Large stones and boulders cover its bottom. Blackfly larvae and pupae were abundant in both of these rivers at the time of treatment.

The temperature of the water in the various streams at time of treatment ranged from 55° F. in Experiment 2, to 63° F. in Experiment 15.

Flow Measurement

The method of flow measurement employed in all but one experiment was as described under Experiment 1 (Aerial Spraying Experiments). The exception was Experiment 10, in which, because of the size of the Warkworth Creek (Figure 8), a somewhat more accurate method was used. This involved the application of Simpson's rule, using the bridge piers as ordinates. Depth was measured at either side of each of the five piers and the flow determined by the float method, two readings being taken at the mid-line of each of the six channels between the banks and the piers.

Methods of Application

The gravity dispenser used in Experiment 3 was contrived by C. N. Husman. It consisted of a 4-gal. drum fitted with a wheel valve laterally near the bottom, leading through five feet of $\frac{3}{8}$ -inch bore rubber tubing to a four-foot length of $\frac{3}{8}$ -inch bore copper pipe. This was arranged as shown in Figure 3, with the container previously calibrated for discharge rate on the bank of the stream, and the outlet of the copper pipe held in a wooden support 2-3 inches above the water surface.

The use of the hand sprayer in Experiment 4 is illustrated in Figure 5. During the first half of the treatment the material was sprayed on to the surface of the stream, with the jet held a few inches above the surface. Some of the finer mist was carried away by the high wind, so during the remainder of the application the jet was held under water. This method appeared quite satisfactory.

In Experiments 5, 6, 7, 9, and 11, the quantities used were so small that application over the required period was made by medicine dropper timed by stop watch. In Experiments 12-15, the water was agitated with a paddle as the insecticide was dribbled in, to ensure quick and uniform distribution. Portable pressure sprayers were used in Experiments 8 and 10 as these involved the treatment of rather large streams (Goose River and Warkworth Creek). The streams were crossed and recrossed by the operator, or operators, as the material was sprayed on.

EFFECTS OF TREATMENTS ON OTHER ANIMAL LIFE IN STREAMS

Effect of Application of DDT by Aeroplane

In Experiment 2, scum consisting of insects, spray material, and debris which had accumulated on vegetation at the margins of the stream in the second aerial spray plot was collected in water on the afternoon of the spraying and preserved in alcohol. Detailed examination of this material was carried out at Edmonton in August, 1947, when its condition was

found to be such that identifications of most insects beyond the family were not practicable. The groups of insects and other arthropods represented,** with the number of specimens of each, are as follows:

Other Arthropods:		Diptera	
Araneae.....	23	Nematocera	
Acarina		Culicidae	
Hydracarinae.....	13	Aedes spp. males.....	2
Insects:		females.....	8
Apterygota		Chironomidae pupae.....	7
Collembola		adults.....	1504
Arthropleona (3 spp.).....	223	Chironomidae	
Symphyleona.....	448	Ceratopogonidae.....	16
Exopterygota		Simuliidae larvae.....	43
Plecoptera		adults.....	3
Nemouridae		Mycetophilidae.....	53
Nemoura spp. nymphs.....	37	Cecidomyiidae.....	9
adults.....	91	Scatopsidae.....	5
Thysanoptera		Brachycera	
Thripidae.....	8	Dolichopodidae.....	1
Homoptera		Empidae.....	13
Aphidae.....	1	Cyclorrhapha	
Charmidae nymphs.....	5	Lonchopteridae.....	2
adults.....	2	Cordyluridae.....	2
Coccidae		Syrphidae.....	6
<i>Steingelia</i> *, n. sp.....	10	Phoridae.....	8
Endopterygota		Chloropidae.....	5
Coleoptera		Anthomyiidae.....	4
Carabidae.....	3	Hymenoptera	
Elateridae.....	3	Chalastogastra	
Dytiscidae larvae.....	2	Tenthredinidae.....	3
adults.....	4	Cimicidae.....	1
Chrysomelidae.....	2	Clistogastra	
Hydroscaphiidae.....	6	Ichneumonidae.....	2
Trichoptera		Mymaridae.....	6
Limnophilidae.....	1	Proctotrupidae.....	4
		Chalcidae.....	6
		Cynipidae.....	2

* Det. by H. Morrison, U.S. Bur. Ent. & Plant Quar., Washington, D.C.

This represents a total of some 2600 specimens belonging to 37 different families, of which only 56 specimens (representing 2 families) belong to species it was desired to control. Of the remainder, many are parasites and predators. Although this was clearly not a strictly random sample, it serves to indicate how insignificant the objective result of such an operation may be in comparison with its total effect on the biocoenotic equilibrium.

Effect of Application of DDT by Hand

In Experiments 3 and 4, wire screens, 3 × 4 feet in size, and 16 mesh to the inch, were fastened in the streams before treatment (DDT 1 : 10,000,000 for 30 minutes) to secure samples of animal life presumably killed by the treatments. Twenty-four hours after treatment the screens were removed and the material collected on them preserved. The quantity of vegetable and inorganic debris mixed in with the specimens in these

** Grateful acknowledgment is made of assistance received from R. B. Miller, E. Moore, and E. H. Strickland, of the University of Alberta, in the identification of material taken in the treated streams.



FIGURE 9. Treatment site of the stream treated in Experiment 12.



FIGURE 10. Drainage channel portion of the stream treated in Experiment 14.

samples was such that it was necessary to remove all the larger fragments by hand for examination, and then to elutriate off the smaller particles for separate examination.

The amount of material obtained from the streams was so great that complete counts were not practicable; a sample was taken by dispersing the specimens in alcohol in petri dishes and making random complete counts on a number of fields of view under a binocular microscope. These counts were then added, and the material as a whole was scrutinized for the occurrence of organisms which did not show up in the sample counts. The results obtained by this method were as follows:

CRUSTACEA		INSECTA	
Branchiopoda		Exopterygota	
Anostraca		Plecoptera	
<i>Branchinecta</i> spp.....	11	Nemouridae	
Cladocera		<i>Nemoura</i> spp. nymphs.....	31
* <i>Eurycercus (glacialis?)</i>	9	adults.....	7
<i>Daphnia</i> spp.....	15	Ephemera nymphs.....	10
Conchostraca		Homoptera	
<i>Limneta</i> spp.....	75	Chermidae.....	2
Copepoda		Endopterygota	
* <i>Heterocope septentrionalis</i>	9	Coleoptera	
Malacostraca		Dytiscidae, larvae.....	4
Amphipoda		adults.....	3
<i>Gammarus</i> spp.....	3	Trichoptera larvae	
ARACHNIDA		Hydropsychidae }.....	10
Araneae.....	1	Rhyacophilidae }.....	16
Hydracarinae.....	2	Other families.....	
		Diptera	
		Nematocera	
		Chironomidae larvae.....	11
		pupae.....	9
		adults.....	35
		Simuliidae, larvae.....	903
		pupae.....	4
		adults.....	35
		Mycetophilidae.....	3
		Brachycera	
		Empidae.....	2

* It is reported that only one previous record of each of these species exists.

Representatives of the following groups were also seen in the general scrutiny: Collembola, Trichoptera (adults), Elateridae, Tenthredinidae, Tipulidae, Anthomyidae, and Mollusca-Pulmonata.

With the exception possibly of the beetles, the material was not in good condition, and more specific identification could not be made.

Sample screens were also used in Experiments 5, 6, and 9, which were carried out in drainage channels.

The number of specimens obtained from the drainage channels was small compared with that from the streams, and there was no difficulty in removing and counting every one. This was probably partly due to the ineffective treatments in Experiments 6 and 9, but also to the much slower current and greater proportion of vegetable matter suspended in the water, screens becoming choked with this material, and hence inoperative, before

many specimens had been caught. The smaller variety of species as compared with material from the streams seems to indicate a less varied fauna. The results obtained are as follows:

CRUSTACEA		INSECTA	
Malacostraca		Exopterygota	
<i>Gammarus</i> sp.....	1	Plecoptera.....	17
Conchostraca		Ephemera nymphs.....	1
<i>Limnetis</i> spp.....	3	adults.....	5
		Endopterygota	
MOLLUSCA		Trichoptera larvae.....	1
Gastropoda		adults.....	2
Pulmonata.....	8	Diptera	
		Nematocera	
		Chironomidae larvae.....	3
		pupae.....	2
		adults.....	2
		Simuliidae larvae.....	10
		pupae.....	6
		adults.....	3
		Tipulidae.....	1
		Brachycera	
		Empidae.....	3

There were many fish in the Warkworth Creek. Twenty-four hours after treatment with DDT at 1 : 6,000,000 for 15 minutes (Experiment 10) fish seemed unaffected. Suckers up to 18 inches in length were seen, and a good catch of pike was made by a fishing expedition two days later. No complaints of dead fish or reduced numbers were received from the local trapper who fed his dogs on the fish caught in traps a short distance upstream.

DISCUSSION

There has been some controversy as to whether DDT and other insecticides actually kill the blackfly larvae in treated streams, or whether they merely cause them to release their hold, possibly to re-attach further downstream, where the concentration may be reduced by the junction of tributaries and the influx of drainage water. In the Churchill locality, the opportunities for such re-attachment are slight, and may only present themselves to larvae in streams draining into the Churchill River. The evidence from this work, however, is that the larvae are actually killed; certainly no living larvae were removed from any sampling screens, and since the exposure period for released larvae moving downstream in the treated water would be greater than that for those caught on the screens or remaining attached, it is unlikely that any of them survive.

The duration of treatment has been recorded as application period and not as exposure period, since the latter naturally increases with the distance from the application site. The concentration, likewise, is that theoretically obtaining at the application site; this figure undergoes a corresponding decrease as the distance from the point of application increases. At any given point downstream the concentration increases gradually to the maximum and decreases again more gradually from the maximum to zero, the rate of both the increase and the decrease being less at greater distances from the site of application. There is no evidence that these factors have any appreciable influence on the mortality obtained for a distance of at least several miles downstream.

The application from the air of 5 per cent DDT in oil solution at 0.26 lb. DDT per acre before the first blackfly pupae were formed, controlled for the season the common species of blackflies breeding in the area sprayed, in streams up to $1\frac{1}{2}$ feet in mean depth. Heavier dosages might be required for deeper streams. It would seem likely that with suitable delivery rates, a single swath applied along the course of the smaller streams, and a swath along either bank with the shore line as swath centre for the larger streams, would prove equally effective, and this would be a practical procedure. Such a procedure, however, would involve greater expense and, judging from the variety of insects killed, a far greater interference with the balance of nature than the more laborious procedure of direct application to the streams. Similar mortality in diverse groups of insects has previously been shown to occur as a result of aerial spraying operations in Panama. Another method of aerial application suitable for treating large rivers, that would minimize the effect on other organisms, would be to lay down successive swaths across the river at a fixed point, timing the applications according to the width of the swaths and the speed of flow, to avoid overlapping them.

Direct application to streams of DDT in oil solutions to give one part DDT to ten million parts of water for an exposure period of 15 minutes caused remarkably little interference with other life, but is somewhat more arduous and might have to be repeated, the first treatment being given immediately before pupation, and the second two or three weeks later. Rarely is there an opportunity to control an economically important insect by using such an efficient, naturally provided, means of distributing an insecticide as the flow of a stream. Advantage should be taken of this opportunity.

The greater residual effect of treatments from the air as compared with ground applications to streams may be the result of continued infiltration of very small amounts of DDT into the stream, washed off rocks, the soil, and to some extent vegetation, by rain and drainage water. Mortality of adults during the spraying operation may be a contributory factor, but since adult females will follow man across the 3-mile width of the Churchill River near its mouth, and related species have been reported to seek a blood meal up to 100 miles from their larval habitat (5), it is unlikely that this factor has much significance after small scale aerial spraying.

Some development of equipment may be desirable for routine direct application stream treatments. The requirements would be a simple form of current meter to enable a ready and reasonably accurate estimate of flow to be made, and a suitable dispenser. For applying a 10 per cent DDT solution in fuel oil, the latter might consist of a stock tank to hold perhaps ten gallons of material, leading to a flow meter consisting of a differential pressure gauge and a variable orifice, controlled by a wheel valve. The dispenser should be calibrated to give the required flow of insecticide at each setting for a concentration of one part DDT in ten million parts of water. The whole apparatus should be designed to fit on a folding tripod stand which could be set up in the bed of the stream, on the bank, or in a small boat. Two men with this equipment and a paddle, stop-watch, measuring-stick, and tape, could treat streams with a flow up to 250 cusecs rapidly and conveniently, and the problem would be

reduced to the ever present one of transportation. A shallow draught boat with outboard motor would assist here, and the motor could be usefully employed also to secure good distribution of insecticide, taking the place of a paddle in the larger streams.

Further work on the lines of that by Fairchild and Barreda (3) to develop a solid formulation of DDT which could be simply allowed to dissolve into the stream might be worth while.

What previous evidence there is seems to indicate that the direct effects of DDT on animals other than insects in ponds and tidewater are unlikely to be serious (7), even at concentrations of one part DDT in three million parts water with exposure time limited only by the stability of the compound. Observations during this work do not entirely support this view; the possibility of accumulated concentrations of DDT in lakes fed by repeatedly treated streams should not be lost sight of. Indirect results can also be serious; blackfly larvae for example, may form a significant food item of fish, especially suckers (1), which are themselves important food of sleigh dogs.

SUMMARY

DDT, chlorinated camphene, chlordane, gamma-benzene hexachloride, and a pyrethrum-piperonyl butoxide preparation were tested in the field for toxicity to the larvae and pupae of *Simulium venustum* Say, and some other species of blackflies.

Against larvae, DDT proved outstanding among these materials, adequate practical control being given by a 15-minute exposure to a concentration of one part of DDT in ten million parts of water, applied in the form of an oil solution, emulsion, or wettable powder. At the concentrations tested, DDT appeared to have no toxic effect on eggs or pupae.

The application of DDT in oil solution by aeroplane at dosages of 0.26 lb. and 0.48 lb. DDT to the acre early in the season controlled blackfly species in streams up to 1½ feet deep flowing through the sprayed area, and prevented reinfestation throughout the remainder of the season.

Data relating to the effect of DDT treatments on other animal species are presented and indicate that far greater interference with the balance of nature may be expected from the aerial than from the ground applications. Suggestions for further investigations are made, and the design of equipment required for routine application of insecticidal liquids to streams is considered.

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NOTES ON SOME OF THE NEWER ACARICIDES¹

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The following brief discussions on some of the newer acaricides used for the control of two-spotted mite, *Tetranychus bimaculatus* Harvey and European red mite, *Paratetranychus pilosus* (C. & F.), are based largely on the greenhouse and insectary experiments described by the junior author in two processed reports published by the Dominion Department of Agriculture: *Report on Acaricide Investigations, 1947, Part I*, issued June 1947; *Part II*, issued December 1947. Unfortunately, the very necessary information about these new materials, which can be obtained only from several years' experience with them in the field under a variety of conditions, is still lacking.

DI-PARA-CHLOROPHENYLMETHYLCARBINOL (DMC)

In greenhouse experiments, DMC at 0.25 lb. per 100 gal. in the form of a 50 per cent spray powder destroyed all active stages and 99 per cent of the eggs of the two-spotted mite. Against European red mite, the mortality two weeks after application was consistently 96 to 100 per cent, the residue remaining highly effective for the two-week period. Both the spray powder and an emulsifiable solution gave very similar results.

DMC was found to be compatible with elemental sulphur, lead arsenate, ferric dimethyl dithiocarbamate, two different fixed copper fungicides, nicotine sulphate, DDT and benzene hexachloride. Hydrated lime and bordeaux mixture slowed down the rate of kill but the ultimate mortality was not affected.

No foliage injury was produced in the greenhouse on bean, tomato, cucumber, roses, apple or plum.

In field experiments DMC gave good results in British Columbia and Washington.

It appears to be specifically an acaricide, having no appreciable insecticidal action on codling moth, aphids, mealy bug and several other greenhouse insects on which it was tried.

No information is on hand regarding its availability and cost, although it is now being advertised under a trade name.

DI-(PARA-CHLOROPHENOXY)METHANE (DCPM)

Used as a 40 per cent micronized spray powder, 1 lb. actual DCPM per 100 gal. was almost 100 per cent effective against both active stages and eggs of the two-spotted mite. This material also has remarkable residual properties against all stages for about a week. Adult mites placed on sprayed plants laid large numbers of eggs before succumbing, but the outstanding residual toxicity of DCPM destroyed nearly 100 per cent of the eggs and young during the first week.

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DCPM is also very effective against the European red mite. In a series of tests it was observed that practically 100 per cent of the population had been destroyed two weeks after spraying with 1 lb. per 100 gal., as a result of the unusual residual and ovicidal effect. At 0.5 lb. per 100 gal., the kill was still over 99 per cent after 14 days, and at 0.25 lb. it was 88 per cent.

DCPM is compatible with all the common spray materials, including hydrated lime and bordeaux.

In one year's field trials against European red mite on apple, plum and peach, DCPM gave quite satisfactory results, although these were not quite so outstanding as in the greenhouse experiments.

In both the greenhouse and orchard experiments, DCPM caused no injury to apple, plum, peach or bean, but stunted cucumbers. Unfortunately, elsewhere it has caused russetting of apples and pears.

With the hope of obviating russetting, investigations are now under way with combinations of low concentrations of DCPM and dinitro-o-cyclohexylphenol (40 per cent spray powder). Preliminary tests indicate that 4.8 oz. DCPM and 1.2 oz. actual DNOCHP, per 100 gal., give good initial kills followed by excellent residual action.

DCPM appears to have relatively low toxicity to higher animals.

PARATHION

Parathion proved to be very effective against the two-spotted mite even when used at 0.15 oz. per 100 gal., destroying 98 per cent of the active forms immediately and 78 per cent after 16 days. However, in order to obtain the full residual value of the material, it was necessary to increase the concentration to 0.6 oz. Comparable results were obtained against European red mite under greenhouse conditions. At high concentrations parathion has pronounced ovicidal action which is profoundly affected by temperature. For example, at 4.8 oz. per 100 gal. the percentage kill of eggs of two-spotted mite at an average temperature of 80.5° F. was 98.2 per cent; at 66.6°, 24.3 per cent; and at 59.6°, 14.8 per cent. The ovicidal effect of low concentrations, e.g. 0.6 oz., is apparently slight, even at high temperatures, and the efficiency of parathion at these rates is dependent on its residual effect on newly-hatched young.

Parathion is compatible with all commonly used spray materials except bordeaux, which, while apparently not greatly affecting the immediate toxicity of the compound, lowers its residual toxicity appreciably. Strangely enough, it is compatible with hydrated lime; parathion-lime spray mixtures allowed to stand 24 hours before use retained their full effectiveness.

Because of its high mammalian toxicity, the future of this material as an acaricide and insecticide now rests in the hands of the toxicologists.

TETRAETHYL PYROPHOSPHATE (TEPP)

Tetraethyl pyrophosphate, including so-called 'hexaethyl tetraphosphate', in which the former is the principal ingredient, is very effective against the active stages of mites, but has no appreciable ovicidal or

residual value, and for this reason the timing of spray applications may be too critical in practical mite control. Uncertainty regarding the actual TEPP content of the experimental materials has been a handicap in all work with this compound.

Recent experiments have demonstrated that a combination of the monoethanolamine salt of DNOCHP and TEPP has outstanding ovicidal value, e.g. mono DNOCHP at 4 oz. actual DNOCHP plus 0.5 pint 'technically pure HETP' per 100 gal. destroyed 98.4 per cent of the eggs of two-spotted mite; mono DNOCHP alone, 19.7 per cent; HETP alone, 4.8 per cent. This may have little practical significance because of cost and also because the combinations may injure foliage of bean and apple.

MONOETHANOLAMINE, TRIETHANOLAMINE AND AMMONIUM SALTS OF DINITRO-O-CYCLOHEXYLPHENOL

These salts were prepared just before use by adding an excess of the respective base to either finely-powdered technical DNOCHP or 40 per cent DNOCHP spray powder. Both forms of DNOCHP were equally satisfactory.

In the greenhouse, all three salts at 2.5 oz. actual DNOCHP per 100 gal. initially destroyed from 94 to 100 per cent of the active forms of European red mite, and examination of the infested plants 14 days after spraying showed a practically complete clean-up by residual action. The salts are not compatible with hydrated lime, bordeaux or certain fixed copper fungicides, and lead arsenate reduces their effectiveness to some extent. They can be used with ferric dimethyl dithiocarbamate, elemental sulphur and some fixed coppers.

In field trials in 1947, promising results were obtained, 2 oz. DNOCHP per 100 gal. being sufficient to give reasonable control of orchard mites in British Columbia, whereas 4 oz. were required in Ontario. In the absence of better materials in commercial quantity, the mono salt is being recommended for growers' use in 1948.

Under greenhouse conditions slight injury was produced on apple foliage, but the salts appear safe in the field. In Ontario they proved to be unsafe on peach, but in British Columbia they caused no injury.

DICYCLOHEXYLAMINE SALT OF DINITRO-O-CYCLOHEXYLPHENOL (DN-111)

Even under greenhouse conditions, DN-111 has given very erratic results. For instance, in the 1946 greenhouse tests against two-spotted mite, the kill at 1.5 lb. per 100 gal. varied from 22.6 to 99.6 per cent, whereas the results from the ammonium salt at approximately equivalent DNOCHP concentrations were remarkably consistent, varying from 93 to 100 per cent.

Field trials with DN-111 in different provinces have also given erratic and generally unsatisfactory results against European red mite on apple. On peach, it has usually been very effective in Ontario.

UNCOMBINED DINITRO-O-CYCLOHEXYLPHENOL

In greenhouse experiments, a 40 per cent DNOCHP spray powder (*DN Dry Mix No. 1*) has given better results, on the whole, than any of the salts, against both the two-spotted mite and the European red mite. At 1.2 oz. actual DNOCHP per 100 gal., it destroyed 98 per cent of European red mite after 14 days, as a result of both immediate and residual toxicity. Where two-spotted mites were placed daily on plants sprayed with 5 oz. DNOCHP per 100 gal., the residue continued to be effective for 10 days, killing 80 to 100 per cent of the introduced mites. In contrast, the mono-ethanolamine salt at equivalent DNOCHP concentrations at the end of 3 days killed 69.8 per cent, and after 7 days only 26 per cent.

At 5 oz. actual DNOCHP per 100 gal., the 40 per cent spray powder produced no injury on apple or bean. On the other hand, finely-powdered technical DNOCHP at the same rate caused extremely severe injury.

LAURYL-2-THIAZOLINYL SULPHIDE

A formulation supplied under the code number IN-4200, at a dilution of 1-800, destroyed 100 per cent of the active stages of two-spotted mite and 98.6 per cent of European red mite. It also killed 91 to 99 per cent of two-spotted mite eggs, and the residual action remained high for approximately 6 days under greenhouse conditions. It appears compatible with most common spray materials, although both hydrated lime and bordeaux slightly reduced the ovicidal action.

Foliage injury, mostly of a minor character, appeared on some of the sprayed plants, including bean and plum; a single series of tests on peach did not produce any injury.

CHLORINATED CAMPHENE

In the greenhouse, chlorinated camphene had considerable acaricide value at high rates, e.g. 1 to 4 lb. per 100 gal. There was little ovicidal action but the residue effectively destroyed the young of two-spotted mite hatching after spraying.

Orchard tests in British Columbia showed this material to be fairly effective against European red mite.

SUMMER OIL

Under conditions where it can be used, 1 per cent summer oil emulsion has proved remarkably efficient over many years. Its chief limitation is its incompatibility with sulphur or DDT. It should be stressed that the summer oil used in Ontario is relatively heavy, with a viscosity of approximately 80 seconds Saybolt at 100° F. and an U.R. of 95 per cent. Quite extensive field experiments with oils of lower viscosity (45 secs. Say.) have shown them to be much inferior as acaricides.

SUMMARY

This paper presents brief notes on the effectiveness and limitations of newer acaricides against European red mite and two-spotted spider mite, based on greenhouse and orchard experiments in Canada. Di-para-chlorophenylmethylcarbinol was one of the best of the specific acaricides. Di-(p-chlorophenoxy)methane was also quite effective and did not injure fruit in limited experiments in Ontario. Parathion was outstanding in greenhouse trials even at very low concentrations. Tetraethyl pyrophosphate lacked residual and ovicidal effects and its value in orchards is doubtful. The grower-prepared monoethanolamine salt of dinitro-o-cyclohexylphenol (DNOCHP) is being extensively used with good results in British Columbia. The proprietary dicyclohexylamine salt of DNOCHP has given very erratic and often unsatisfactory results. Uncombined DNOCHP was a good acaricide but there may be danger of foliage injury. Lauryl-2-thiazolinyI sulphide was promising in preliminary tests. Chlorinated camphene was fairly effective. Summer oil emulsion remains one of the best acaricides but is incompatible with sulphur or DDT.

All acaricides tested possessed limitations of varying degree.

FLAXSEED MUCILAGE AND ITS EFFECT ON THE FEEDING VALUE OF LINSEED OIL MEAL IN CHICK RATIONS¹

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In contrast with most of the common sources of plant protein supplements, flaxseed has a characteristically high mucilage content. In earlier work 6.3 parts of mucilage were obtained from 100 parts of flaxseed (8). This work also showed that the mucilage was not attacked by the enzymes in dried pancreas and that when fed to guinea pigs and rats about 75 per cent was excreted unchanged. Recent work has indicated that it is the salt of a polymerised aldoteuronic acid (1), (9). It is stated that by virtue of its capacity to absorb water the mucilage contributes to the regularity of the digestive system in large animals and hence accounts for the beneficial effects of linseed oil meal in live stock rations (7). In poultry rations, however, the laxative effect due presumably to the presence of the mucilage might become harmful when they contain 4 to 5 per cent of linseed oil meal (10). At higher levels, this effect should become more pronounced. Also, the high viscosity of aqueous solutions of the mucilage causes the ration to become sticky and leads to the development of beak necrosis and deformity. This is a characteristic symptom in chicks fed large amounts of linseed oil meal and could be one reason for the reduced feed intake and poor growth. If on the other hand the digestibility of the mucilage in chicks is low, as in the case in rats, the presence of this viscous and relatively indigestible material in the small intestine might interfere with the normal processes of digestion and absorption. It was thought that this specific detrimental effect of the mucilage could be tested by feeding a standard ration containing added mucilage.

Many workers have found that the feeding quality of linseed oil meal is improved by a process of water treatment with and without subsequent drying of the moistened meal (2), (3), (4), (5), (6). Various drying temperatures have been used but it has not been reported whether or not the drying temperature has any specific effect. The present work was undertaken to study this effect and also the effect of these various treatments on the viscosity of the mucilage. If the mucilage content of linseed oil meal influences the feeding value of the latter it may be expected that any treatment which affects the feeding value of the linseed oil meal would also alter the physical characteristics of the mucilage.

Methods and Materials

EXPERIMENT I

An aqueous solution of the mucilage was prepared by soaking flaxseed in four times its weight of water at room temperature for 24 hours and centrifuging. The mucilage was precipitated by pouring the above extract

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into twice its volume of 90 per cent alcohol, washed with a little absolute alcohol, air dried and then ground finely. Analysis gave 11.5 per cent protein as calculated from total nitrogen by the Kjeldahl method. This evidently was a water soluble impurity carried over from the flaxseed but further purification was not attempted. The product was added at 2 per cent level to a standard starter ration. This ration was prepared by adding 5 lb. yellow corn and 15 lb. soybean meal to a basal ration of the following composition: Ground yellow corn 13 lb.; ground wheat 20 lb.; ground barley 20 lb.; ground oats 13 lb.; buttermilk powder 2 lb.; fish meal (65 per cent protein) 1 lb.; meat meal (50 per cent protein) 2 lb.; alfalfa meal 5 lb.; dried brewer's yeast 1 lb.; ground limestone 2 lb.; iodised salt 1 lb.; fortified fish oil (1850 A—400D₃) 200 gm.; manganese sulphate 6 gm., and riboflavin 0.1 gm. On the basis that flaxseed contains about 6.5 per cent of mucilage and 35 per cent of oil and that the prepared mucilage contains about 10 per cent of protein matter as impurity the above addition would be equivalent to an inclusion of 18 per cent linseed oil meal in terms of mucilage content. The original starter ration before and after admixture with mucilage was fed for a 5 weeks' period using 15 chicks per lot. Results are given in Table 1.

TABLE 1.—EFFECT OF ADDING FLAXSEED MUCILAGE TO A STANDARD CHICK STARTER RATION

Lot	Mucilage added to ration	Initial body weight, gm.	Final body weight, gm.	Feed gain ratio
1	Nil	58.8	305.8	2.7
2	2 per cent	58.5	224.9*	3.5

* The difference between final weights in the two lots is highly significant.

Results and Discussion

From Table 1 it is seen that the addition of mucilage depressed the feeding value of the ration when measured by gain in body weight and efficiency of feed utilisation. Almost all the birds in the lot receiving the mucilage ration had beak necrosis and many had crooked beaks and ruffled feather coats which are characteristic symptoms of feeding high levels of linseed oil meal. The lessened feed intake which was in part due to the beak defects would be one reason for the poor growth. But the feed: gain ratio indicates that the presence of the mucilage also depressed the utilisation of the feed actually consumed. Some of the factors responsible for the low feeding value of linseed oil meal would thus appear to be present in the mucilage portion. It is possible that aqueous extraction and precipitation with alcohol brought about some alteration in the nature of the mucilage and that in its native state its effect would be different. It is also possible that the protein material carried over in the mucilage preparation contained some specific toxic constituent present in the flaxseed.

*Methods and Materials***EXPERIMENT II**

Three portions of a sample of hexane extracted linseed oil meal were soaked in four times the amount of water at room temperature for ten hours and dried at room temperature, 60-70 degrees C. and 90-100 degrees C., respectively. The original and treated meals were added at about 20 per cent level on a protein equivalent basis to the basal ration used in the previous experiment. The crude protein content of the rations were equalised by adjusting the amounts of corn. Results of the feeding trial using 15 chicks per lot for a four-week period are given in Table 2.

Results and Discussion

From Table 2 it is seen that drying the water incubated meal at 90-100 degrees C. gave a product significantly better than all the others. The improvements which resulted from the lower drying temperatures, however, did not quite reach the significance level. The mortality in these two lots and in the lot which received the untreated meal was rather high. This might indicate that there is a larger residual toxicity in the meals prepared at lower drying temperatures. In contrast with the results in this experiment, Kratzer (2) observed significant improvement in the meal by incubation with water followed by drying at room temperature. This difference might be due to the fact that he used a higher level (35 per cent) of the meal and the rations contained little or no animal protein, whereas 5 per cent of animal protein supplements was used in this experiment. It would thus appear that, apart from the improvement brought about by the action of water during the soaking period, the temperature of drying is a factor which affects the degree of improvement. Within limits, a higher drying temperature may be expected to give a better product.

TABLE 2.—EFFECT OF DRYING TEMPERATURE ON THE FEEDING VALUE OF WATER TREATED LINSEED OIL MEAL

Lot	Description of linseed oil meal	Initial body weight, gm.	Final body weight, gm.	Mortality per cent	Significance levels* Lots:		
					1	2	3
1	Untreated	60.5	132.6	33.3	—	—	—
2	Incubated with water, dried at room temperature	60.6	160.6	20.0	0.07	—	—
3	Incubated with water, dried at 60-70 degrees C.	60.5	163.3	33.3	0.06	—	—
4	Incubated with water, dried at 90-100 degrees C.	60.0	194.1	6.7	0.01	0.02	0.05

* The significance level of differences between final weights in any two lots is obtained by cross reference. The value for lots 2 and 4 is thus 0.02.

*Methods and Materials***EXPERIMENT III**

An aqueous solution of flaxseed mucilage was prepared as in Experiment I and divided into four parts. One portion was kept at room temperature for 24 hours, a second portion was autoclaved for 15 minutes at 250 degrees F., cooled and brought back to original weight with water, a third portion was dried in the oven at 80 degrees C., water added to redissolve the mucilage and brought back to original weight with water. The fourth portion was used immediately as control. The viscosity of these liquids was compared by noting the time of flow of a definite volume of the liquid under similar conditions through a vertically fixed tube of narrow and uniform bore. Results are given in Table 3.

Results and Discussion

It is seen from Table 3 that the time of flow and hence the viscosity of the mucilage is lowered by all the treatments, particularly by autoclaving and by drying. This effect may arise through changes in the colloidal nature of the solution. Water treatment of linseed oil meal with subsequent drying would therefore be expected to cause similar changes in the mucilage of linseed oil meal. In a similar way this may account for the varying degrees of improvement observed in the feeding trial in Experiment II.

TABLE 3.—VISCOSITY CHANGES IN AN AQUEOUS SOLUTION
OF FLAXSEED MUCILAGE

No.	Sample	Time of flow in seconds
1	Untreated	915
2	Kept at room temperature for 24 hours	485
3	Autoclaved	122
4	Dried at 80° C. and redissolved	179

SUMMARY AND CONCLUSIONS

1. Flaxseed mucilage when incorporated into a starter ration containing 15 per cent soybean meal has a significant growth depressing effect on chicks and causes the development of beak necrosis.

2. In the improvement of linseed oil meal by water treatment, higher drying temperature has a greater beneficial effect.

3. The viscosity of aqueous solutions of flaxseed mucilage is appreciably reduced by autoclaving and by drying at 80 degrees C., and to a lesser extent by holding at room temperature for 24 hours.

4. It is suggested that the presence of the mucilage in linseed oil meal is one of the factors involved in its low feeding value for the chick and that the improvement in the meal as a result of higher drying temperatures in the water treatment process is partly due to alteration in physical properties, notably viscosity, of the mucilage.

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CYANOGENETIC GLUCOSIDES AND TRYPSIN INHIBITORS IN LINSEED OIL MEAL¹

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Many workers have reported that linseed oil meal gives unsatisfactory results when used at levels over 5 per cent in chick starter rations (1), (5), (11), (12). Even after correcting amino acid deficiencies in the meal by adequate supplementation it caused significant growth depression in chicks (9). When it was used at 30 per cent levels in the ration high mortality and intestinal disorder were observed (3). These results have been taken as indicative of the presence of certain toxic factors in the meal. There is some evidence that linseed oil meal in the ration depresses the availability of some of the B-complex vitamins (6). It is believed also that other factors might be involved, namely, cyanogenetic glucosides and trypsin inhibitors. The presence of these factors in some vegetable feed-stuffs is known to cause unsatisfactory results under certain conditions of feeding practice.

Flaxseed contains a glucoside which under favourable conditions of warmth and moisture liberates prussic acid by the action of an enzyme present in the seed (10). However, no specific instance of prussic acid poisoning has been reported in poultry. Ordinarily, the heat to which the ground seed is subjected during the oil expressing process inactivates or destroys the enzyme. Also, the addition to chick rations of potassium cyanide in amounts equivalent to the prussic acid content in some linseed oil meal samples did not lead to any poisoning effect.* It is possible, however, that different samples of the meal differ in the extent to which they liberate prussic acid.

The presence and properties of trypsin inhibiting factors have been extensively studied in the case of soybean (7), (8) (13), (14). These factors interfere with the normal action of tryptic enzymes in the digestive system and in this way depress the utilisation of the protein in the food. It would appear to be fairly well established that their presence partly accounts for the unsatisfactory feeding value of raw soybean for chicks. In view of the above reports it was felt that the possible implication of these two toxic factors should be more fully investigated in linseed oil meal.

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EXPERIMENT I

Methods and Materials

Three samples of linseed oil meal were used in this experiment, one being an expeller sample† designated (*c*) and the other two being hydraulic samples* designated (*f*) and (*h*). The concentration of hydrocyanic acid (free and combined) in these samples was determined according to the A.O.A.C. method of analysis for content of cyanogenetic glucosides in feedstuffs (2). Since in two of the samples studied the maximum liberation of hydrocyanic acid was obtained after four hours of maceration in water prior to steam distillation, this period was used instead of the recommended two-hour period. A feeding trial was also conducted on these samples of linseed oil meal. Week-old Barred Plymouth Rock chicks, 15 per lot, were placed on three starter rations containing the three samples of the meal at about 10 per cent level in the ration. The basal mixture consisted of the following ingredients per 100 lb.: ground yellow corn 20 lb.; ground wheat 20 lb.; ground barley 20 lb.; ground oats 13 lb.; buttermilk powder 2 lb.; fish meal (65 per cent protein) 2 lb.; meat meal (50 per cent protein) 3 lb.; soybean meal 1 lb.; alfalfa meal 5 lb.; dried brewer's yeast 1 lb.; ground limestone 2 lb.; iodized salt 1 lb.; fortified fish oil (1850 A-400D₃) 200 gm.; manganese sulphate 6 gm., and riboflavin 0.1 gm. Results of the experiment are given in Table I.

TABLE 1.—CYANOGENETIC GLUCOSIDES IN LINSEED OIL MEAL SAMPLES AND GROWTH RESPONSE OF CHICKS ON RATIONS CONTAINING THESE SAMPLES AT 10 PER CENT LEVEL

No.	Sample	Hydrocyanic acid per cent (free and combined)	Mean chick weight at 6 weeks, in gm.
1	Expeller (<i>c</i>)	0.004	199.2
2	Hydraulic (<i>f</i>)	0.038	250.0
3	Hydraulic (<i>h</i>)	0.050	291.4

Results and Discussion

It is seen from Table I that as the content of hydrocyanic acid (free and combined) in the samples increases their feeding value does not decrease. The expeller sample which gave the poorest results in the feeding trial yielded only traces of hydrocyanic acid. It is possible that the enzyme responsible for the liberation of hydrocyanic acid in this case had been inactivated by the relatively higher temperatures involved in the expeller process. Though the feeding quality of the meals would be determined by other factors as well—and hence they are not strictly comparable on the basis of the yield of hydrocyanic acid—this experiment would indicate that the importance of the cyanide content in the toxic effect of the meal is relatively small if existent at all.

† Kindly supplied by Victory Mills Ltd., Toronto.

* Kindly supplied by Canada Linseed Oil Mills Ltd., Montreal.

TABLE 2.—ENZYME HYDROLYSIS OF CASEIN IN PRESENCE OF RAW AND AUTOCLAVED AQUEOUS EXTRACTS OF LINSEED OIL MEAL AND OF FLAXSEED (EXPRESSED AS ML. OF 0.2 N ALKALI FOR FORMOL TITRATION)

Sample	Time of hydrolysis		
	3 hr.	7 hr.	17 hr.
(a) Casein plus enzyme	10.40 (10.65)*	11.70 (12.10)	13.45 (13.90)
(b) Enzyme alone	3.55 (3.70)	3.80 (3.85)	3.85 (4.05)
(c) Casein plus raw extract plus enzyme	10.50 (11.20)	11.90 (12.75)	13.65 (15.20)
(d) Raw extract plus enzyme	4.25 (4.20)	4.50 (4.35)	4.90 (4.65)
(e) Casein plus autoclaved extract plus enzyme	10.60 (10.90)	12.00 (12.45)	14.05 (14.40)
(f) Autoclaved extract plus enzyme	4.40 (4.15)	4.60 (4.10)	5.10 (4.30)
Casein alone (a-b)	6.85 (6.95)	7.90 (8.25)	9.60 (9.85)
Casein in presence of raw extract (c-d)	6.25 (7.00)	7.40 (8.40)	8.75 (10.55)
Casein in presence of autoclaved extract (e-f)	6.20 (6.75)	7.40 (8.35)	8.95 (10.10)

* The figures within brackets are values for flaxseed extracts. The other figures are those for linseed oil meal extracts.

Methods and Materials

EXPERIMENT II

A sample of linseed oil meal was prepared by extracting powdered flaxseed with petroleum ether in order to avoid the use of high temperatures. The extracted meal was mixed with ten times the amount of water, the pH adjusted at 4.0, the mixture kept overnight at 5 degrees C. and then centrifuged to obtain the clear aqueous extract. A portion of this extract was autoclaved for 15 minutes at 250 degrees F., cooled and made up to the original weight with water. The other portion was used in the raw state. Two mixtures were prepared, each containing 50 ml. of a 6 per cent casein solution, 10 ml. of 15 per cent di-sodium phosphate solution, 0.2 gm. of a dried pancreas preparation* of reported activity of 3 U.S.P. Units (1 : 75), and 1 ml. of toluene. To one mixture was added 20 ml. of the raw aqueous extract of linseed oil meal and to the other was added 20 ml. of the autoclaved extract. These mixtures were then incubated at 37 degrees C. and the pH maintained at 8.5 during the hydrolysis. Three blanks were run simultaneously under the same conditions, one containing the enzyme alone, another containing the enzyme with the raw extract and the third containing the enzyme with the autoclaved extract. The progress of the

*Kindly supplied by the Viobin Corporation, Monticello, Ill.

hydrolysis was measured by formol titration of 20 ml. aliquots at definite intervals. In a second experiment an aqueous extract of flaxseed was prepared in a manner similar to the preparation of the linseed oil meal extract, but the weight of water used was four times that of the flaxseed. A portion of the extract was autoclaved and the hydrolysis of casein in presence of the raw and autoclaved extracts was conducted in the same way as before. Results of both experiments are given in Table 2.

Results and Discussion

In Table 2 the extent of hydrolysis in casein alone and in casein in presence of the raw and autoclaved extracts, respectively has been calculated by subtracting the corresponding blanks. It is seen that the addition of the raw extracts (of flaxseed and linseed oil meal) had no appreciable effect on the hydrolysis of casein. Autoclaving the extracts likewise had little effect. The aqueous extract from uncooked soybean meal in a similar experiment was found to depress the trypsin hydrolysis of casein to a value as low as about one-third the original and this inhibition was destroyed on autoclaving the extract (13). The presence in linseed oil meal and in flaxseed of trypsin inhibiting factors similar to those present in soybean meal has therefore not been shown under these experimental conditions. "In vitro" trials of this type, however, may not identify all the enzyme inhibiting factors which in the digestive system of the chick would interfere with normal digestion.

The content of trypsin inhibitors in various feedstuffs has been reported since this work was undertaken (4). According to the data submitted, flaxseed does not contain these factors.

SUMMARY AND CONCLUSION

1. Data obtained on three samples of linseed oil meal indicate that increases in the yield of hydrocyanic acid liberated under incubation are not associated with corresponding decreases in the feeding value of the meals in chick rations.

2. The presence of raw aqueous extracts from linseed oil meal, and from flaxseed which should have contained the trypsin inhibiting factors, has little influence on the tryptic hydrolysis of casein. Autoclaved extracts likewise are without noticeable effect. These findings suggest that trypsin inhibitors are not present in these extracts to any appreciable extent.

3. It is concluded that the observed toxic effect of linseed oil meal in chick rations is due primarily to factors other than cyanogenetic glucosides and trypsin inhibitors.

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BOOK REVIEWS

JESSEN'S *BOTANIK DER GEGENWART UND VORZEIT*. Offset reprint edition. 495 pp. Chronica Botanica Co., Waltham, Mass.; Thorburn and Abbott, Ltd., Ottawa, Canada. Price \$6.00.

This is a reprint of a history of botany originally published in 1864, and forms Volume 1 of a new series, entitled "Pallas," to be published by the Chronica Botanica Co., consisting of reprint editions of out-of-print and classic scientific works.

PRÉCIS DES DÉCOUVERTES SOMIOLOGIQUES, by C. S. Rafinesque. Reprinted from the original (1814) edition by Peter Smith, 321 Fifth Avenue, New York 16, N.Y. Introduction by E. D. Merrill, Arnold Professor of Botany and Director of the Arnold Arboretum of Harvard University. 1948. \$4.00.

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- Index to Trade of Canada (Imports) entered for consumption, 1946.
 Grain and feed review, December, 1936 and August, 1943.
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